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Abstract

Moringa oleifera is an edible medicinal plant used to fight malnutrition in Africa. In this study, *M. oleifera* flowers, fruits and seeds from Guinea-Bissau were characterized for their nutritional composition and hydroethanolic and aqueous extracts were prepared to investigate the phenolic profiles and bioactivities. Seeds presented higher levels of proteins (~31 g/100 g dw), fat (~26 g/100 g dw) and flavan-3-ol derivatives, while carbohydrates, proteins, citric acid, and glycosylated flavonoids were abundant in fruits and flowers, these last samples also being rich in α-tocopherol (~18 mg/100 g dw). Some of the identified polyphenols had never been described in *M. oleifera*. In general, hydroethanolic extracts contained more polyphenols and were more active against lipid peroxidation, NO production, and tumour cells growth. Significant antimicrobial effects against the tested bacteria and fungi strains were displayed by both hydroethanolic and aqueous extracts. The *M. oleifera* potential to fight malnutrition and health issues was highlighted.

Keywords: *Moringa oleifera*; famine food; nutritional composition; phenolic profile; antioxidant/anti-inflammatory activity; cytotoxicity.

1. Introduction

The search for plants and plant-based products that can face the raising necessities of food and medicines in a context of climate changes and food scarcity is nowadays a major challenge in Africa where persist malnutrition problems (Muyonga et al., 2016). In this context, *Moringa oleifera* Lam. (Moringaceae) appears as a species with nutritional, medicinal and agronomic value. This fast-growing, deciduous tree is native to the Indian subcontinent and Pakistan, and has become naturalized in the tropical and subtropical areas around the world, namely in many African countries due to its easy adaptability and tolerance to a wide range of environmental conditions regarding climate and soil (Daba, 2016).

M. oleifera is one of the most auspicious plants used as a suitable alternative for preventing and alleviating malnutrition challenges, especially hidden hunger health issues (Padayachee & Baijnath, 2019). It is considered to be a "Miracle tree" or "Tree of life" due to the substantial beneficial effects that it has on health, but also due to its potential use in water sanitation and environmental conservation (Daba, 2016). *M. oleifera* preparations have been reported in the scientific literature as having a wide range of pharmacological properties, including antimicrobial, hypotensive, hypoglycemic, immunomodulatory, and anti-inflammatory activities. In addition, all *M. oleifera* parts (including leaves, fruits, seeds, pods, and flowers) have been used in traditional foods and dishes for human consumption (Daba, 2016).

The leaves and seeds are eaten fresh, powdered or cooked and contain a varied profile of nutrients and health-promoting compounds, such as fatty acids, tocopherols, β-carotene, and phenolic compounds. The fruits are fibrous and traditionally used to treat digestive problems and prevent colon cancer. Flower extracts, in turn, are used in culinary preparations to enhance the taste and colour of dishes (Padayachee & Baijnath, 2019; Ziani et al., 2019). These *M. oleifera* organs are also known to be good sources of secondary metabolites, including terpenoids, flavonoids, tannins, anthocyanins, and proanthocyanidins (Ajibade et al., 2013).

These bioactive compounds contribute to the therapeutic and medicinal properties of *M. oleifera* and may justify its uses by the indigenous system of medicine in the treatment of common ailments and disorders, such as anaemia, asthma, diarrhea, skin infections, headaches, swelling, hysteria, cholera, scurvy, respiratory disorders, diabetes, cough, sore throat, and chest congestion (Padayachee & Baijnath, 2019). Therefore, this edible medicinal plant appears as a natural remedy easily accessible to populations in developing countries that need basic healthcare, especially in areas where Western medicine is inaccessible or expensive (Padayachee & Baijnath, 2019). Curiously, *M. oleifera* seed powder is used as a purifying agent in the treatment of water, being able to eliminate pathogenic bacteria up to 99%, whereas fresh leaves can be used to extract a juice used as a growth hormone (or soil fertilizer) able to increase crop yields by 25-35% (Daba, 2016).

In Guinea-Bissau (West Africa), the awareness of local populations about the medicinal and nutritional properties of *M. oleifera* has increased in the last years, where the trade of seeds and dried and crushed leaves is under development. Despite this, the exploitation of the different edible and medicinal parts of this plant in this country is far to reach their full potential (Bancessi et al., 2019). Therefore, due to the multiple traditional uses and applications of *M. oleifera*, this study was performed to determine the detailed nutritional and chemical composition (proximate constituents, free sugars, organic acids, tocopherols, fatty acids, and phenolic compounds) of seed, flower and fruit samples collected in two distinct locations in Guinea-Bissau using official methods of food analysis and advanced chromatographic techniques. In addition, the antioxidant, anti-inflammatory, cytotoxic, and antimicrobial activities of hydroethanolic, infused and decocted extracts prepared with the three *M. oleifera* organs were assessed *in vitro* using different cellular assays and food-borne microorganisms. In this way, it is intended to demonstrate and validate the food and medicinal potential of *M.*

oleifera, which can have a direct impact on the food security of local African populations and be useful for the development of new functional foods and nutraceuticals.

2. Material and methods

2.1. Sampling and samples preparation

M. oleifera seeds, flowers and immature fruits (**Fig. 1**) were collected in early May 2019 in two locations in Guinea-Bissau: Granja (11º 52'02''N; 15º 36'06''W), a state farm inside Bissau urban area, and in a homegarden in Ponta Romana, Quinhamel, located in the countryside (11º54'18''N; 15º49'45''W). The two collecting sites are about 30 km apart and the soil and climatic conditions in both sites are similar (ferralsols, rainfall c. 1500 mm per year). The main differences are the urban vs. rural environment and the fact that in Granja the harvested trees were isolated, with direct sunlight during most of the day and in Ponta Romana the samples were taken from trees of a living fence in a homegarden, with less direct sunlight. The plant samples were then lyophilized (FreeZone 4.5, Labconco, MO, USA) and reduced to a fine powder that was stored in well-sealed plastic bags at -20 ºC in the dark until further analysis.

2.2. Nutritional value and energy assessment

The *M. oleifera* edible samples were analysed for moisture, protein, fat, and ash contents following the AOAC analytical procedures (AOAC International, 2016). Total carbohydrates were calculated by difference and the energetic value was calculated according to the Regulation (EC) No. 1169/2011 of The European Parliament and of the Council as follows: energy (kcal/100 g dried weight (dw)) = 4 × (g protein + g carbohydrate) + 9 × (g fat).

2.3. Chromatographic analysis of free sugars, organic acids, fatty acids, and tocopherols

Free sugars were analysed in a high-performance liquid chromatography (HPLC) system (Knauer, Smartline system 1000, Berlin, Germany) coupled to a refractive index detector (Smartline System 1000), using the internal standard (melezitose, Sigma-Aldrich, St Louis, MO, USA) method previously described by Spréa et al. (2020). Data were recorded and processed using Clarity 2.4 software (DataApex, Prague, Czech Republic) and the results were expressed as g per 100 g dw.

Organic acids were analysed by ultra-fast liquid chromatography (Shimadzu 20A series, Shimadzu Corporation, Kyoto, Japan) coupled to a diode-array detector operating in the conditions described by Spréa et al. (2020). The compounds were identified by comparing their retention time and UV-Vis spectra with those of standards (oxalic, malic, ascorbic, citric, and fumaric acids, Sigma-Aldrich, St. Louis MO, USA) and quantified based on calibration curves obtained by plotting the peak area recorded at 245 nm for ascorbic acid and at 215 nm for the remaining acids against concentration. Data were recorded and processed using LabSolutions Multi LC-Photodiode Array (PDA) software (Shimadzu Corporation, Kyoto, Japan) and the results were given as g per 100 g dw.

The fatty acids profile was determined by gas-liquid chromatography (DANI 1000, Switzerland) coupled to a flame ionization detector (FID) operating in the conditions previously described by Spréa et al. (2020). Data were recorded and processed using Clarity 4.0 software and the results were given as relative percentage of each fatty acid.

Tocopherols were determined using the internal standard (tocol, Matreya, Pleasant Gap, PA, USA) method and the HPLC system (Smartline System 1000, Knauer, Berlin, Germany) coupled to a fluorescence detector (FP-2020, Jasco, Easton, USA) programmed for excitation at 290 nm and emission at 330 nm, as previously described by Spréa et al. (2020). Data were

recorded and processed using Clarity 2.4 software and the results were given as mg per 100 g dw.

2.4. Preparation of *M. oleifera* hydroethanolic and aqueous extracts

The *M. oleifera* seed, flower and immature fruit samples were prepared in hydroethanolic, infused and decocted extracts to evaluate their composition in phenolic compounds and the *in vitro* bioactive properties. These preparation/extraction methods were selected according to the traditional uses of the different parts of the plant (Dhakar et al., 2011; Ilyas et al., 2015; Lim, 2014).

To prepare the hydroethanolic extracts, each sample (2 g) was mixed with ethanol/water solution (80:20, *v/v*; 30 mL) and stirred for 1 h at room temperature. After filtering the supernatant through Whatman filter paper No 4, the residue was re-extracted and the combined filtrates were concentrated under reduced pressure (rotary evaporator Büchi R-210, Switzerland) at 40 °C and the aqueous phase was subsequently lyophilized (Iyda et al., 2019).

For decoctions, each sample (2 g) was boiled with distilled water (100 mL) for 5 min in heating plate (VELP Scientific) and then filtrated through Whatman filter paper No 4. The obtained decoctions were frozen and lyophilized (Iyda et al., 2019).

Only seeds and flowers were used to prepare infusions. The samples (2 g) were infused with freshly boiled distilled water (100 mL), left aside for 5 min and subsequently filtered through Whatman filter paper No 4. The resulting extracts were frozen and lyophilized (Adouni et al., 2018).

2.5. HPLC-DAD-ESI/MS^n analysis of phenolic compounds

Phenolic compounds were analysed in hydroethanolic, infused and decocted extracts, which were redissolved in ethanol/water (80:20, *v/v*) and water, respectively, to a final concentration

of 10 mg/mL and filtered using 0.22 µm disposable filter disks. The analysis was performed in a HPLC system (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, California, USA) coupled with a diode-array detector (DAD, using 280 and 370 nm as preferred wavelengths) and a Linear Ion Trap (LTQ XL) mass spectrometer (MS, Thermo Finnigan, San Jose, CA, USA) equipped with an electrospray ionization (ESI) source. Separation was made in a Waters Spherisorb S3 ODS-2 C18 column (3 µm, 4.6 mm × 150 mm; Waters, Milford, MA, USA). The operating conditions were previously described by Bessada, Barreira, Barros, Ferreira, and Oliveira (2016), as well as the identification and quantification procedures. The results were given as mg per g of extract.

2.6. Evaluation of bioactive properties *in vitro*

2.6.1. Antioxidant activity

Two cell-based assays were performed to measure the *in vitro* antioxidant activity of the extracts (0.1563–5 mg/mL), following methodologies formerly described by Spréa et al. (2020) and Lockowandt et al. (2019). The extracts capacity to inhibit the formation of thiobarbituric acid reactive substances (TBARS) was assessed using porcine brain cell tissues as oxidizable substrates, and the results were expressed as half maximal effective extract concentration (EC_{50}) values (mg/mL). The oxidative haemolysis inhibition assay (OxHLIA) was performed to assess the extracts capacity to protect sheep erythrocytes from the AAPH (2,2′-azobis(2-methylpropionamidine) dihydrochloride)-induced oxidative haemolysis. Half maximal inhibitory concentration (IC_{50}) values (µg/mL) were calculated for time intervals (Δt) of 60 and 120 min and translate the extract concentration required to keep 50% of the erythrocyte population intact for 60 and 120 min. Trolox (Sigma-Aldrich, St. Louis, MO, USA) was used as a positive control.

2.6.2. Nitric oxide (NO)-production inhibition activity

The anti-inflammatory activity of the extracts (at concentrations up to 400 µg/mL) was assessed based on the nitric oxide (NO) production by a lipopolysaccharide (LPS)-stimulated murine macrophage (RAW 264.7) cell line. The NO production was quantified based on the nitrite concentration using the Griess Reagent System kit containing sulphanilamide, *N*-1-naphthylethylenediamine dihydrochloride and nitrite solutions, following a procedure previously described by Corrêa et al. (2015). Dexamethasone (Sigma-Aldrich, St. Louis, MO, USA) was used as a positive control, while no LPS was added in negative controls. The effect of the tested extracts in NO basal levels was also assessed by performing the assay in the absence of LPS. The results were expressed as IC_{50} values (µg/mL), which correspond to the extract concentration providing 50% of NO production inhibition.

2.6.3. Cytotoxic activity

The extracts cytotoxicity was assessed by the sulforhodamine B (Sigma-Aldrich, St. Louis, MO, USA) assay against four human tumour cell lines (acquired from Leibniz-Institut DSMZ), namely MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma), and HepG2 (hepatocellular carcinoma), following a protocol previously described by Spréa et al. (2020). Ellipticine (Sigma- Aldrich, St. Louis, MO, USA) was used as a positive control. The same assay was also used to evaluate the hepatotoxicity of the extracts against a non-tumour cell line (PLP2, porcine liver primary cells) obtained as described by Spréa et al. (2020). The extract concentration (µg/mL) causing 50% cell growth inhibition (GI_{50}) was calculated and used to express the results.

211 *2.6.4. Antimicrobial activity*

212 The extracts were redissolved in 5% dimethyl sulfoxide (DMSO) to a concentration of 10
213 mg/mL and further diluted. The microdilution method (Soković et al., 2010) was performed to
214 assess the antimicrobial activity against the Gram-negative bacteria *Escherichia coli* (ATCC
215 35210), *Salmonella* Typhimurium (ATCC 13311) and *Enterobacter cloacae* (ATCC 35030),
216 and the Gram-positive *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (clinical isolate)
217 and *Listeria monocytogenes* (NCTC 7973). The antifungal activity was assessed against
218 *Aspergillus fumigatus* (ATCC 1022), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus niger*
219 (ATCC 6275), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC
220 9112), and *Penicillium aurantiogriseum* (food isolate) (Corrêa et al., 2015). The minimum
221 extract concentrations that completely inhibited bacterial growth (MICs) were determined by a
222 colorimetric microbial viability assay, and minimum bactericidal concentration (MBC) and
223 minimum fungicidal concentration (MFC) were also calculated. Streptomycin, ampicillin,
224 ketoconazole and bifonazole (Sigma-Aldrich, St. Louis, MO, USA) were used as positive
225 controls, and 5% DMSO was used as a negative control.

226

227 2.7. Statistical analysis

228 Three samples were used for each analysis and all the assays were carried out in triplicate. The
229 results were presented as mean values and standard deviation. A Student's *t*-test was applied to
230 assess significant difference among plant samples with a different geographic origin
231 (Quinhamel and Bissau), with $\alpha = 0.05$. In the bioactive assays, a one-way analysis of variance
232 (ANOVA) was applied, followed by Tukey's HSD test, with $\alpha = 0.05$, to assess significant
233 differences between hydroethanolic, infused and decocted extracts. The analysis was carried
234 out using SPSS v. 22.0 program SPSS Statistics software (IBM Corp., Armonk, NY, USA).

235

3. Results and discussion

3.1. Nutritional composition of *M. oleifera* edible parts

Since the plants composition is affected by different factors, such as the edaphoclimatic conditions of the different growing sites, agricultural practices, harvesting period, and genetic characteristics, among others (Iyda, Fernandes, Calhelha, et al., 2019), the studied samples of *M. oleifera* were collected at two distinct locations in Guinea-Bissau. **Table 1** presents the proximal composition of the *M. oleifera* seeds, flowers, and fruits collected in Quinhamel and Bissau. Carbohydrates were found to be major constituents in all studied samples; the highest levels were detected in the fruit (71.91±0.04 and 79.6±0.1 g/100 g dw) and the lowest in the seeds (38.85±0.03 and 41.2±0.3 g/100 g dw in samples from Bissau and Quinhamel, respectively). Proteins rank second with the seeds showing the higher levels (30.0±0.6 – 31.88±0.08 g/100 g dw), followed by the flower and the fruit. These last two plant parts also had an interesting content of ash (total minerals), which ranged from 19.83±0.01 to 21.3±0.4 g/100 g dw. As expected, the seeds had a higher fat content (~26.3 g/100 g dw) than the other two edible parts of *M. oleifera*. In addition, fruits collected in Quinhamel stood out with a significantly higher fat content (4.3±0.1 g/100 g dw) than those collected in Bissau (2.67±0.06 g/100 g dw). The results obtained in this study are slightly lower than those previously reported by Gopalakrishnan, Doriya, and Kumar (2016) and Liang, Wang, Li, Chu, and Sun (2019) for the fat (38.67 and 39.12 g/100 g) and protein (35.97 and 40.34 g/100 g) contents in Indian *M. oleifera* seeds, but were higher for carbohydrates (8.67 and 8.94 g/100 g).

Regarding the energy contribution, 100 g fruit and flower portions provide comparable values (~390–396 kcal), while that of seeds were higher (~518–522 kcal) mainly due to the fat content. According to previous reports, *M. oleifera* oil can accelerate wound healing (Liang et al., 2019) and the seed protein fraction has potential to be used in surface water purification due to coagulant effects (Baptista et al., 2017). Therefore, *M. oleifera* edible parts arise as interesting

possibilities for being exploited as raw materials for production of vegetable oil, protein-rich foods and skincare products.

As shown in **Table 1**, the chromatographic analysis allowed to detect and quantify four free sugars in the studied *M. oleifera* flowers and fruits, namely fructose, glucose, sucrose and trehalose, while just glucose and fructose were found in the seeds. The highest levels were quantified in the fruits (16.7±0.1 – 18.8±0.2 g/100 g fw), followed by the flowers (11.1±0.1 – 12.0±0.2 g/100 g fw) (**Fig. S1,** supplementary material) and lastly by the seeds with significantly lower levels (1.32±0.09 – 1.86±0.06 g/100 g fw). It was also noted that the quantitative sugar profile of the fruit and flower samples seemed to have been affected by their different origin. These differences could be attributed to edaphoclimatic factors and some biotic conditions that can affect biochemical and physiological processes involved in the plant sugars' production (Ziani et al., 2019). In a previous study, Ziani et al. (2019) identified fructose, glucose and sucrose in *M. oleifera* leaves from Algeria and reported a total free sugars content of 3.82 g/100g dw. Upadhyay, Yadav, Mishra, Sharma, and Purohit (2015) described *L*-arabinose, *D*-galactose, *D*-glucuronic acid, *L*-rhamnose, *D*-mannose, and *D*-xylose as the predominant sugars in the purified whole-gum exudates of *M. oleifera*.

Regarding organic acids, the analysis allowed identifying oxalic, malic, ascorbic, citric, and fumaric acids in flower and fruit samples from both locations (**Table 1**). Citric and malic acids were the major compounds, while just traces of fumaric acid were detected. Fruits collected in Bissau contained a higher level of ascorbic acid (0.65±0.02 g/100 g fw) than those from Quinhamel or the flower samples. The total organic acid contents ranged from 4.71±0.02 – 5.75±0.02 g/100 g fw in fruits to 5.85±0.01 – 6.42±0.01 g/100 g fw in flowers. In *M. oleifera* seeds, ~10.5 g/100 g fw of oxalic acid were quantified (**Table 1**), about twice the total content of organic acids found in the other two parts of the plant. Traces of fumaric acid were also detected. It is known that plant foods with a high oxalic acid concentration should be consumed

moderately, because the high intake of oxalates may promote the formation of kidney stones, irritation of the intestinal mucosa, and also interferes with calcium absorption (Iyda, Fernandes, Ferreira, et al., 2019). To the best of the authors' knowledge, no data are available in the literature regarding the organic acid composition of *M. oleifera* seeds, flowers or fruits. In leaves, Ziani et al. (2019) already reported oxalic, malic and ascorbic acids.

The main fatty acids identified in the studied *M. oleifera* edible parts are also presented in **Table 1**, while the detailed profiles are shown in **Table S1** provided in Supplementary Material. Twenty-one fatty acids were identified in the fruit and flower lipid fractions, while just 14 were detected in the seed samples. The flower lipid fraction was mainly composed by unsaturated fatty acid (SFA; ~41%, due to the contribution of C16:0, C22:0 and C18:0), followed by polyunsaturated fatty acids (PUFA; 32.4±0.2 – 37.9±0.1 %), namely α-linolenic (C18:3n3) and linoleic (C18:2n6) acids. *M. oleifera* fruits were abundant in monounsaturated fatty acids (MUFA; 49.0±0.1 – 55.0±0.6 %), particularly those collected in Quinhamel homegardens, due to the high contents of oleic acid (C18:1n9), followed by SFA (31.3±0.2– 33.4±0.5 %), which predominated in the fruit samples from Bissau, given the high levels of palmitic (C16:0), behenic (C22:0) and stearic (C18:0) acids. MUFA also predominated in the seed samples (73.1±0.5 – 75.1±0.2 %), mostly C18:1n9 but also minor levels of eicosenoic (C20:1) and palmitoleic (C16:1) acids. The SFA C16:0 and C22:0 were also detected in this plant part. In a previous work, Zheng et al. (2019) studied the effects of soil drenching and foliar spraying of boron on *M. oleifera* seed oil quality and reported C18:1 levels ranging from 64.24 to 71.17%, a result comparable to that obtained in the present study (69.44±0.4 and 71.6±0.2% for seeds from Bissau and Quinhamel, respectively). The lipid composition of *M. oleifera* seeds is greater than that of soybean, which makes it nutritionally important and the refined seed oil is acceptable to substitute the olive oil because of the presence of all the essential fatty acids in it (Singh et al., 2019).

311 The tocopherols composition of the studied *M. oleifera* edible parts is shown in **Table 1**, where
312 it can be seen that *α*-tocopherol was the prevalent isoform in all samples, followed by *δ*-
313 tocopherol. The flower samples showed the highest *α*-tocopherol concentrations, ranging from
314 17.22±0.09 to 18.90±0.01 mg/100 g dw (HPLC profile in **Fig. S2**, supplementary material).
315 Fruit and seed samples revealed a total content of tocopherols ranging from 2.71±0.01 to
316 4.86±0.03 g/100 g dw and the samples collected in Bissau showed higher levels of these
317 lipophilic antioxidants. Singh et al. (2020) reported that tocopherols together with ascorbic acid,
318 carotenoids and flavonoids are antioxidants found in *M. oleifera* with the ability to eliminate
319 reactive oxygen species.
320
321 *3.2.* Polyphenols compositions of *M. oleifera* hydroethanolic and aqueous extracts
322 Data on the chromatographic characteristics (retention time, UV-Vis spectra in the maximum
323 absorption, molecular ion, and main MS^2 fragments) and tentative identification of the phenolic
324 compounds found in the hydroethanolic, infused and decocted extracts of *M. oleifera* are
325 described in **Table 2**. Twenty-four phenolic compounds were found, being 19 glycosylated
326 flavonol derivatives, 3 phenolic acids, and 2 flavan-3-ols. The phenolic composition of *M.*
327 *oleifera* has been extensively studied by other authors (Makita et al., 2016; Nouman et al., 2016;
328 Ramabulana et al., 2016; Ziani et al., 2019); however, there are many compounds identified in
329 the present work that, to the best of the author's knowledge, have never been previously
330 identified in *M. oleifera*. Peaks **3**, **6**, **13**, **14**, **15**, **18**, **20**, **21**, and **22** were identified as (+)-
331 catechin, (-)-epicatechin, quercetin-3-*O*-rutinoside, apigenin-6-*C*-glucoside, quercetin-3-*O*-
332 glucoside, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside, kampferol-3-*O*-glucoside,
333 and isorhamnetin-3-*O*-glucoside, respectively, by comparing their retention time, UV-Vis
334 spectra, and mass fragmentation patterns with those of the available commercial standards.
335 Only three phenolic acids were tentatively identified, peaks **1/2** ($[M-H]^-$ at *m/z* 337) and **4** ([M-

H]$^-$ at *m/z* 367), as *cis/trans* 3-*O*-*p*-coumaroylquinic acid and 3-*O*-feruloyquinic acid, respectively. Peak **1** presented a base peak at *m/z* 191 (quinic acid) along with a peak at *m/z* 163 (corresponding to the *p*-coumaroyl acid moiety); peak **2** presented the same chromatographic behaviour, leading to the respective identification of the *cis* and *trans* isomers of *p*-coumaroylquinic acid. These peaks (**1/2** and **4**) have been previously identified in the foliar parts of *M. oleifera* from South Africa, after being exposed to certain levels of radiation (Ramabulana et al., 2016).

The flavonoid was, without any doubt, the most abundant group of phenolic compounds identified in studied *M. oleifera* samples, with glycosylated derivatives of quercetin having a superior numerical expression to any other identified flavonoid aglycone. Peaks **10** ([M-H]$^-$ at *m/z* 625), **16** ([M-H]$^-$ at *m/z* 505), and **17/19** ([M-H]$^-$ at *m/z* 549), tentatively identified as quercetin-*O*-dihexoside, quercetin-*O*-acetylhexoside and quercetin-malonylhexoside, respectively, have been previously identified in the leaves of *M. oleifera* from South Africa (Ramabulana et al., 2016), Pakistan (Nouman et al., 2016), and Namibia (Makita et al., 2016). Peak **5** presented a pseudomolecular ion [M-H]$^-$ at *m/z* 711, and MS^2 fragments at *m/z* 667 (loss of 44 u, carboxyl radical), *m/z* 505 (loss of sinapoylradical), *m/z* 463 (loss of sinapoyl and acetyl radicals), and *m/z* 301 (quercetin aglycone), which allowed the tentative identification as quercetin-acetylglucoside-sinapic acid. This peak has not been identified in *M. oleifera* samples, so its tentative identification was performed following the previously described by Medina et al. (2017) in *Passiflora edulis* shell, without numbering the oxygen atoms and radicals position since it was not possible to compare the abundance of each fragment. Peak 11, also a glycosylated derivative of quercetin, presented a pseudomolecular ion [M-H]$^-$ at *m/z* 595, and MS^2 fragments at *m/z* 463 and *m/z* 301, corresponding to the loss of a pentosyl and hexosyl moieties, respectively. As peak **5**, peak **11** was not previously identified in *M. oleifera* samples, so its tentative identification followed the previously described by Barros et al. (2013) in *Cistus*

ladanifer. The second major flavonoid group was that of *C*-glycosylated apigenin derivatives, represented by peaks **7** ([M-H]$^-$ at *m/z* 593), **9** ([M-H]$^-$ at *m/z* 593) and **12** ([M-H]$^-$ at *m/z* 431), tentatively identified as apigenin-6,8-*C*-diglucoside, apigenin-*O*-hexoside-*C*-hexoside, and apigenin-*C*-hexoside, respectively, following the previously described by Truchado et al. (2011) and Qiao et al. (2011), being previously identified similar compounds in *M. oleifera* leaves (Nouman et al., 2016; Ramabulana et al., 2016). Kaempferol derivatives were also found; peak **8**, tentatively identified as kaempferol-*O*-malonylhexoside, was previously reported in *M. oleifera* leaf samples by Makita et al. (2016), and peak **23**, presenting a pseudomolecular ion [M-H]$^-$ at *m/z* 695, was tentatively identified as kaempferol-*O*-malonyldihexoside, following the previously described by Sánchez-Salcedo et al. (2016) in *Morus* spp. leaves (to the best of the authors' knowledge, this peak as not been described previously in *M. oleifera*). Finally, peak **24**, tentatively identified as isorhamnetin-*O*-malonylhexoside, was previously described in *M. oleifera* leaves by Ziani et al. (2019).

Data on the quantification of the phenolic compounds present in *M. oleifera* edible parts are presented in **Table 3**. The profile of phenolic compounds present in each group of *M. oleifera* samples was very different, quantitatively but also qualitatively, with very few similar compounds between samples, which could be explained by the different physiological function of the studied plant parts and/or different microenvironmental conditions in each sampling site, namely a wetter and more shaded environment at the Ponta Romana homegarden.

The hydroethanolic extracts prepared with flowers from Bissau presented the highest total concentration of phenolic compounds, 14.7±0.1 mg/g of extract, followed by the Quinhamel flower hydroethanolic extract, with 13.8±0.1 mg/g of extract. The seed samples were the only ones presenting flavan-3-ols derivatives, representing the major group of phenolics within this group. Another information that is important to highlight is the fact that the decoction prepared with the Quinhamel fruit sample had no phenolic compounds. Although an aqueous preparation

386 such as decoction can lead to the thermal degradation of compounds, the absence of compounds
387 may be related to the sample itself, since the hydroethanolic extract of this sample also had the
388 lowest total concentration of phenolic compounds (0.765±0.001 mg/g extract) within the
389 corresponding group of samples.
390 Despite the very different phenolic profile, the most abundant phenolic compound (apart from
391 seeds samples) was peak 1 (*cis* 3-*O*-*p*-coumaroylquinic acid), which did not produce an effect
392 of higher concentration of phenolic acids, since it was the group of flavonoids that stood out
393 (less in the Quinhamel flower hydroethanolic extract). These results are in accordance with the
394 described by Ziani et al. (2019) and Nouman et al. (2016) in *M. oleifera* leaves, in which they
395 revealed total concentrations of flavonoids of up to 30 mg/g extract and 2.98 mg/g extract,
396 respectively.
397
398 *3.3. Bioactive properties of M. oleifera* hydroethanolic and aqueous extracts
399 To evaluate the bioactive properties of the different *M. oleifera* edible parts, hydroethanolic,
400 infused and decocted extracts were prepared according to traditional uses and applications.
401 Fruits are traditionally prepared as a culinary vegetable, stewed in curries and soups. In India
402 and Bangladesh, fruits are usually prepared by boiling pods to the desired level of tenderness
403 in a mixture of coconut milk and spices (Lim, 2014). Therefore, only hydroethanolic and
404 decocted extracts were prepared in this study with the fruit samples. On the other hand, seeds
405 and flowers were used to prepare hydroethanolic, infused and decocted extracts. Traditionally,
406 mature seeds are fried and eaten like peanuts in Nigeria and added to sauces for their bitter taste.
407 In Pakistan are used to prepare *M. oleifera* seed tea infusions (Ilyas et al., 2015) and in India
408 seed decoctions (Dhakar et al., 2011). The flowers are cooked and consumed either mixed with
409 other foods or fried in batter, butter or oil. In West Bengal and Bangladesh, these are usually

410 cooked with green peas and potato, while in Africa are eaten as a vegetable, added to sauces or
411 used to make infusions (Lim, 2014).
412
413 *3.3.1. Antioxidant activity*
414 Two *in vitro* cell-based assays were used to measure the antioxidant activity of the
415 hydroethanolic, infused and decocted extracts of the different *M. oleifera* parts (**Table 4**). These
416 assays evaluate the extract ability to inhibit the formation of thiobarbituric acid reactive
417 substances (TBARS) and the oxidative haemolysis (OxHLIA) using porcine brain tissues and
418 erythrocytes as oxidizable biological substrates, respectively. As can be observed in **Table 4**,
419 in the TBARS assay, significant differences were found between the three plant parts and
420 between the extraction methods. The hydroethanolic extracts showed the lowest EC_{50} values,
421 thus translating a greater capacity to inhibit the TBARS formation than the aqueous extracts.
422 This result could be justified by the greater efficiency of the hydroethanolic mixture in
423 extracting phenolic compounds and other antioxidants (Padayachee & Baijnath, 2019). In the
424 OxHLIA assay, the sheep erythrocytes were subjected to the haemolytic action of both
425 hydrophilic and lipophilic radicals generated in *in vitro* by the thermal decomposition of the
426 free-radical initiator AAPH and as a consequence of the initial attack, respectively. By
427 observing the data presented in **Table 4**, it can be noticed that infusions prepared with seed and
428 flower samples from Bissau showed the best results, with IC_{50} values lower than those of the
429 trolox, the water-soluble analog of vitamin E used as a positive control. Interestingly, the
430 hydroethanolic extracts did not show any antihemolytic effect. In a previous study, Pakade,
431 Cukrowska, and Chimuka (2013) compared the antioxidant activity of *M. oleifera* leaves and
432 flowers to that of several vegetables from South Africa, including spinach, cauliflower,
433 broccoli, cabbage, and peas, and reported a total flavonoid content in *M. oleifera* three times

434 higher than that quantified in the others plant foods, thus concluded that *M. oleifera* is a better
435 source of antioxidants.
436
437 *3.3.2. NO-production inhibition activity*
438 The NO-production inhibition (or anti-inflammatory) activity of the tested *M. oleifera* extracts
439 was assessed based on the NO-production inhibition activity and the results are presented in
440 **Table 4**. The extracts prepared with the seed samples from both locations were able to reduce
441 the production of NO by LPS-stimulated murine macrophages. This result followed the same
442 trend observed for the TBARS formation inhibition assay, with the hydroethanolic preparations
443 showing the best results. However, flower and fruit extracts did not reveal anti-inflammatory
444 activity at the tested concentrations. In previous studies, Minaiyan, Asghari, Taheri, Saeidi, and
445 Nasr-Esfahani (2014) showed that hydroalcoholic seed extracts are effective in the treatment of
446 experimental colitis and associated this effect with the major bioactive biophenols and
447 flavonoids (Minaiyan et al., 2014). In turn, Jaja-Chimedza et al. (2017) connected the anti-
448 inflammatory and antioxidant properties of *M. oleifera* seeds to the presence of isothiocyanates.
449 Accordingly Padayachee and Baijnath (2020), infusions of *M. oleifera* leaves, seeds, flowers,
450 roots, and bark display anti-inflammatory activity. Alhakmani, Kumar, and Khan (2013) also
451 attributed anti-inflammatory effects to the *M. oleifera* flower extract, which supports the
452 traditional use of this preparation in Oman and other Asian countries.
453
454 *3.3.3. Cytotoxicity to tumour and non-tumour cells*
455 Considering the described uses of the different parts of *M. oleifera* in traditional medicine, the
456 prepared extracts were also tested for their cytotoxicity for tumour and non-tumour cell lines.
457 The performed sulforhodamine B assay allows to evaluate the effect of the extracts on cell
458 proliferation (Ziani et al., 2019). Therefore, GI_{50} values translate the extract concentration

providing 50% of cell growth inhibition. As presented in **Table 4**, the hydroethanolic extracts of seed and flower samples originated the lower GI_{50} values, thus translating a higher activity than the aqueous extracts against HeLa (cervical), HepG2 (hepatocellular), MCF-7 (breast) and NCI-H460 (lung) tumour cells. Among the hydroethanolic extracts, those prepared with seeds were more effective against the HepG2 cell line, regardless of the geographic origin of the samples (with GI_{50} of 82±5 – 95±2 µg/mL), while those prepared with flowers were more cytotoxic to breast MCF-7 cells (with GI_{50} of 163±5 – 187±10 µg/mL). For seeds, the decoctions proved to be the least cytotoxic preparations for the tested cell lines (given the higher GI_{50} values), which is in line with the results obtained with the OxHLIA assay (where they also had the highest IC_{50} values). The aqueous flower extracts were not cytotoxic at the tested concentrations, nor any of those prepared with the fruits.

In previous studies, Jung (2014) found that aqueous *M. oleifera* leaf extracts are able to reduce the proliferation and invasion of cancer cells by inducing apoptosis, inhibiting the tumour cell growth and decreasing the level of internal reactive oxygen species in human lung cancer cells. Al-Asmari and co-workers (2015) evaluated the anticancer properties of *M. oleifera* leaf, bark and seed extracts against breast (MDA-MB-231) and colorectal (HCT-8) cancer cells and obtained remarkable anticancer activities with the leaf and bark extracts, while the seed extract showed less activity. It has also been reported that the flavonoids quercetin and kaempferol present in *M. oleifera* extracts may act as potential chemopreventive agents, being able to reduce the proliferation of human carcinoma through the induction of *in vitro* apoptosis (Padayachee & Baijnath, 2019). In addition, the presence of these and other antioxidants in *M. oleifera* allows to reduce oxidative stress and, consequently, help prevent the development of cancer. Among the metabolites with antioxidant activity found in *M. oleifera* are flavonoids, phenolic acids, saponins, tannins, β-carotene, and terpenoids (Singh et al., 2019).

Table 4 also shows that, with the exception of the hydroethanolic seed extracts, no other extract was cytotoxic to the non-tumour PLP2 cells at the tested concentrations. This toxicity of the hydroethanolic seed extracts to porcine liver primary cells may somehow justify the absence of antihemolytic activity in the OxHLIA assay, since the erythrocytes may have been rapidly lysed due to the cytotoxic effect of these hydroalcoholic preparations.

In many countries, *M. oleifera* seed powder is used to purify water on aquaculture farms due to its coagulation properties. Nevertheless, the application of a large amount of this ingredient in aquaculture ponds leads to fish mortality due to the presence of toxic or antinutritional compounds. The seed powder toxicity has already been observed in guppies (*Poecilia reticulata*), Nile tilapia (*Oreochromis niloticus*), protozoa (*Tetrahymena pyriformis*), and bacteria (*Escherichia coli*) (Kavitha et al., 2012). Regarding ethanolic and aqueous extracts of both *M. oleifera* fruits and leaves, Luqman, Srivastava, Kumar, Maurya, and Chanda (2011) showed that these are well tolerated by experimental animals without toxicity of the extracts up to a dose of 100 mg/kg of body weight. The aqueous and hydroethanolic extracts of *M. oleifera* flowers have also been described as having a significant hepatoprotective effect, which may be due to the presence of quercetin, a well-known flavonoid with hepatoprotective activity (Upadhyay et al., 2015). Furthermore, Singh et al. (2020) described that alcoholic and aqueous extracts from flowers and roots of *M. oleifera* act as hepatoprotectors against the effect of acetaminophen (a drug used to treat pain and fever) by decreasing the level of serum enzymatic markers and bilirubin levels.

3.3.4. Antimicrobial activity

The results of the antimicrobial activity of *M. oleifera* extracts are presented in **Table 5**. All the extracts had significant antimicrobial effects against the tested bacteria and fungi. The MIC and MBC values obtained for *Bacillus cereus*, *Staphylococcus aureus, Listeria monocytogenes*, and

Escherichia coli, as well as for *Enterobacter cloacae* and *Salmonella* Typhimurium, were comparable to those of streptomycin and ampicillin, the antibiotics used as positive controls, thus translating a similar bacteriostatic and bactericidal activity. In general, decoctions were the most effective preparations against the tested bacteria and, in the case of seeds, it is also worth noting the higher activity of the hydroethanolic and infused extracts prepared with seeds from Quinhamel and the decocted extracts made with seed from Bissau. The antimicrobial activity of *M. oleifera* leaf, root, bark and seed extracts against bacteria, yeasts, dermatophytes, and helminths pathogenic to human was previously investigated by Upadhyay, Yadav, Mishra, Sharma, and Purohit (2015), which verified that the seed aqueous extract inhibits the growth of *Pseudomonas aeruginosa* and *S. aureus*. According to previous reports, the antimicrobial activity of *M. oleifera* seed powder is conferred by a short cationic protein (Singh et al., 2019), as well as by saponins, tannins, phenolics, and alkaloids (Padayachee & Baijnath, 2019).

The antifungal activity of the tested *M. oleifera* extracts resulted in MIC and MBC values lower or similar to those of the positive controls ketoconazole and bifonazole (**Table 5**). The antifungal activity of aqueous leaf extracts of *M. oleifera* was previously confirmed by Padayachee and Baijnath (2020) against *Penicillium* spp., while the ethanolic extract also inhibited *Candida albicans*, *Penicillium* spp., and *Mucor* spp. The phytochemical screening of this plant part revealed the presence of alkaloids, flavonoids, saponins, terpenoids, steroids, tannins, and cardiac glycosides, which may act as natural antimicrobials (Padayachee & Baijnath, 2019; Raj et al., 2011).

4. Conclusion

The results of the present study highlighted the nutritional quality of *M. oleifera* fruits, seeds and flowers from Bissau and Quinhamel and the bioactive potential of their herbal preparations. These edible and medicinal matrices stood out not only with high nutritional value, but also for

533 their potential to be used in food fortification and in the development of new functional foods,
534 nutraceuticals and pharmaceutical formulations. *M. oleifera* is a natural resource to be valorised
535 by underprivileged population facing poverty and malnutrition issues, but also by other
536 stockholders, specifically in underdeveloped and developing nations that have an insufficient
537 technical resources.
538
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548
549 **Declaration of competing interest**
550 The authors declare that they have no known competing financial interests or personal
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552
553 **References**
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683 **Figure captions**

684 **Fig. 1**. Edible parts of *Moringa oleifera* characterized in this study: a) flowers; b)

685 seeds; and c) Immature fruits.

686

687

688 **Supplementary material captions**

689 **Table S1.** Detailed fatty acid composition of *M. oleifera* edible parts.

690 **Fig. S1**. Free sugars profile of *M. oleifera* fruits from Bissau characterized in this study:

691 1- Mobile phase; 2- Fructose; 3- Glucose; 4- Sucrose; 5- Melezitose (PI).

692 **Fig. S2**. Tocopherols profile of *M. oleifera* flowers from Quinhamel characterized in this

693 study: 1- Mobile phase; 2- α-Tocopherol; 3- δ-Tocoperol; 4- Tocol (PI).

Table 1

Nutritional value and composition in free sugars, organic acids, main fatty acids, and tocopherols of *M. oleifera* edible parts.

	Seeds		Student's *t*-test	**Flowers**		Student's *t*-test	**Fruits**		Student's *t*-test
	Quinhamel	**Bissau**	*p*-value	**Quinhamel**	**Bissau**	*p*-value	**Quinhamel**	**Bissau**	*p*-value
Moisture (%)	np	np	-	81.4±0.5	81.4±0.1	0.851	79.0±0.4	76.8±0.9	0.006
Fat (g/100 g)	26.0±0.1	26.6±0.1	0.001	5.27±0.07	5.02±0.05	0.002	4.3±0.1	2.67±0.06	<0.001
Proteins (g/100 g)	30.0±0.6	31.88±0.08	0.002	21.3±0.4	19.83±0.01	0.001	19.79±0.04	19.49±0.06	0.476
Ash (g/100 g)	2.8±0.1	2.67±0.01	0.001	7.93±0.09	7.95±0.07	0.346	6.31±0.06	5.93±0.05	<0.001
Carbohydrates (g/100 g)	41.2±0.3	38.85±0.03	<0.001	65.5±0.3	67.2±0.1	0.001	79.6±0.1	71.91±0.04	<0.001
Energy (kcal/100 g)	518.3±0.4	522.2±0.5	<0.001	394.6±0.5	393.2±0.1	0.007	396.3±0.5	389.7±0.3	<0.001
Fructose (g/100 g)	nd	nd	-	2.19±0.02	1.51±0.01	<0.001	3.00±0.04	2.86±0.04	0.003
Glucose (g/100 g)	0.15±0.05	0.16±0.04	0.651	6.01±0.07	3.30±0.04	<0.001	8.02±0.04	10.03±0.08	<0.001
Sucrose (g/100 g)	1.17±0.04	1.70±0.03	<0.001	2.93±0.09	5.52±0.07	<0.001	5.03±0.04	4.92±0.01	0.005
Trehalose (g/100 g)	nd	nd	-	0.82±0.03	0.75±0.01	0.005	0.63±0.01	1.01±0.05	<0.001
Total sugars (g/100 g)	1.32±0.09	1.86±0.06	<0.001	12.0±0.2	11.1±0.1	0.001	16.7±0.1	18.8±0.2	<0.001
Oxalic acid (g/100 g)	10.44±0.05	10.6±0.2	0.153	0.77±0.01	1.82±0.01	<0.001	0.66±0.01	1.18±0.01	<0.001
Malic acid (g/100 g)	nd	nd	-	1.79±0.02	1.29±0.02	<0.001	1.84±0.03	1.30±0.01	<0.001
Ascorbic acid (g/100 g)	nd	nd	-	0.25±0.01	0.19±0.01	<0.001	0.35±0.01	0.65±0.02	<0.001
Citric acid (g/100 g)	nd	nd	-	3.05±0.01	3.12±0.02	0.001	1.84±0.02	2.62±0.01	<0.001
Fumaric acid (g/100 g)	tr	tr	-	tr	tr	-	tr	tr	-
Total organic acids (g/100 g)	10.44±0.05	10.6±0.2	0.153	5.85±0.01	6.42±0.01	<0.001	4.71±0.02	5.75±0.02	<0.001
C16:0	6.1±0.2	7.0±0.2	0.002	19.7±0.1	21.6±0.2	<0.001	12.8±0.2	10.4±0.2	<0.001
C18:0	5.53±0.06	6.5±0.2	<0.001	4.64±0.01	4.23±0.09	<0.001	4.67±0.06	4.73±0.07	0.221
C18:1n9	71.6±0.2	69.4±0.4	<0.001	25.8±0.1	20.32±0.01	<0.001	52.4±0.6	48.8±0.1	<0.001
C18:2n6	0.65±0.03	0.69±0.06	0.192	15.1±0.1	14.4±0.5	0.023	7.42±0.08	8.5±0.3	<0.001
C18:3n3	0.21±0.02	0.195±0.005	0.116	16.4±0.1	22.3±0.3	<0.001	6.67±0.09	6.3±0.4	<0.001
C22:0	7.0±0.2	6.98±0.09	0.446	5.6±0.2	5.4±0.5	0.414	7.43±0.09	9.1±0.1	<0.001
C24:0	1.43±0.08	1.33±0.01	0.039	6.0±0.3	5.0±0.3	0.005	1.67±0.09	3.6±0.2	<0.001
SFA (%)	24.1±0.2	26.0±0.4	0.001	41.0±0.4	40.84±0.08	0.366	31.3±0.2	33.4±0.5	<0.001
MUFA (%)	75.1±0.2	73.1±0.5	0.001	26.6±0.1	21.23±0.04	<0.001	55.0±0.6	49.0±0.1	<0.001
PUFA (%)	0.86±0.01	0.89±0.06	0.299	32.4±0.2	37.9±0.1	<0.001	14.4±0.2	17.5±0.6	<0.001
α-Tocopherol (mg/100 g)	2.22±0.02	3.36±0.01	<0.001	18.90±0.01	17.22±0.09	<0.001	3.13±0.05	4.67±0.02	<0.001
δ-Tocopherol (mg/100 g)	0.48±0.01	1.48±0.03	<0.001	2.08±0.01	2.68±0.07	<0.001	0.45±0.04	0.19±0.01	<0.001
Total tocopherols (mg/100 g)	2.71±0.01	4.84±0.01	<0.001	20.98±0.01	19.90±0.01	<0.001	3.58±0.09	4.86±0.03	<0.001

np - not performed; nd - not detected; tr – traces; C16:0 - palmitic acid; C18:0 - stearic acid; C18:1n9 - oleic acid; C18:2n6 - linoleic acid; C18:3n3 - *α*-linolenic acid; C22:0 - behenic acid; C24:0 - lignoceric acid; SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids.

Table 2

Phenolic compounds identified in *M. oleifera* edible parts. It is presented the retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), and mass spectral data.

Peak	Rt (min)	$\lambda_{max\ (nm)}$	$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification	Reference/method used for quantification
1	6.19	311	337	191(8), 173(6), 163(100), 153(3), 119(5)	*cis* 3-*O*-*p*-Coumaroylquinic acid	Ramabulana et al. (2016)
2	7.09	311	337	191(8), 173(6), 163(100), 153(3), 119(5)	*trans 3-O-p*-Coumaroylquinic acid	Ramabulana et al. (2016)
3	7.11	280	289	245(25), 203(10), 137(31)	(+)-Catechin	Standard compound
4	7.16	323	367	193(100), 191(5), 173(5), 149(3), 134(8)	3-*O*-Feruloyquinic acid	Ramabulana et al. (2016)
5	8.6	256/268/351	711	667(52), 505(100), 463(37), 301(21)	Quercetin-*O*-acetylglucosyl-sinapic acid	Medina et al. (2017)
6	9.57	280	289	245(100), 205(52), 151(29), 137(37)	(-)-Epicatechin	Standard compound
7	9.97	322	593	575(11), 503(24), 473(100), 383 (12), 353(27)	Apigenin-6,8-*C*-diglucoside	Truchado et al. (2011)
8	12.59	342	695	651(53), 489(100), 447(28), 285(41)	Kaempferol-*O*-malonyldihexoside	Sánchez-Salcedo et al. (2016)
9	13.55	337	593	473(35), 431(100), 353(5), 311(62), 283(5)	Apigenin-*O*-hexoside-*C*-hexoside	Qiao et al. (2011)
10	15.05	359	625	301(100)	Quercetin-*O*-dihexoside	Nouman et al. (2016)
11	15.98	350	595	463(31), 301(100)	Quercetin-*O*-pentoside-*O*-hexoside	Barros et al. (2013)
12	16.51	334	431	413(5), 341(6), 311(100)	Apigenin-*C*-hexoside	Nouman et al. (2016)
13	17.77	354	609	301(100)	Quercetin-3-*O*-rutinoside	Standard compound
14	18.35	337	431	413(7), 341(26), 311(100)	Apigenin-6-*C*-glucoside	Standard compound
15	18.91	353	463	301(100)	Quercetin-3-*O*-glucoside	Standard compound
16	20.19	353	505	463(30),301(100)	Quercetin-*O*-acetylhexoside	Ramabulana et al. (2016)
17	20.21	352	549	505(12), 463(22), 301(100)	Quercetin-*O*-malonylhexoside	Makita et al. (2016)
18	21.06	347	593	285(100)	Kaempferol-3-*O*-rutinoside	Standard compound
19	22.06	350	549	505(72), 463(27), 301(100)	Quercetin-*O*-malonylhexoside	Makita et al. (2016)
20	22.07	353	623	315(100)	Isorhamnetin-3-*O*-rutinoside	Standard compound
21	22.39	346	447	285(100)	Kampferol-3-*O*-glucoside	Standard compound
22	23.36	352	477	315(100)	Isorhamnetin-3-*O*-glucoside	Standard compound
23	24.62	346	533	489(89), 447(10), 285(100)	Kaempferol-*O*-malonylhexoside	Makita et al. (2016)
24	25.92	353	563	519(88), 315(100)	Isorhamnetin-*O*-malonylhexoside	Ziani et al. (2019)

Table 3

Content (mg/g extract) of the phenolic compounds identified in hydroethanolic, infused and decocted extracts of *M. oleifera* edible parts.

Peak	Seeds						Flowers						Fruits			
	Quinhamel			Bissau			Quinhamel			Bissau			Quinhamel		Bissau	
	HyEth	Inf	Dec	HyEth	Inf	Dec	HyEth	Inf	Dec	HyEth	Inf	Dec	HyEth	Dec	HyEth	Dec
1	nd	nd	nd	nd	nd	nd	4.7±0.1^{a}	1.214±0.01^{e}	1.443±0.003^{d}	3.86±0.02^{b}	1.93±0.03^{c}	0.61±0.01^{f}	nd	nd	0.50±0.01^{g}	0.20±0.01^{h}
2	nd	nd	nd	nd	nd	nd	0.39±0.01*	nd	nd	0.471±0.00*	nd	nd	nd	nd	nd	nd
3	0.178±0.002^{a}	0.035±0.001^{c}	0.10±0.01^{b}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.08±0.01*	0.030±0.003*
5	nd	nd	nd	nd	nd	nd	0.23±0.01^{a}	0.092±0.001^{c}	0.14±0.02^{b}	0.020±0.002^{d}	0.02±0.01^{d}	tr	nd	nd	nd	nd
6	0.44±0.02^{a}	0.081±0.004^{d}	0.07±0.01^{e}	0.10±0.02^{c}	0.28±0.01^{b}	0.29±0.01^{b}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	nd	nd	nd	nd	nd	nd	0.254±0.001^{c}	0.051±0.003^{f}	0.161±0.001^{d}	0.69±0.02^{a}	0.39±0.01^{b}	0.15±0.01^{e}	nd	nd	nd	nd
8	nd	nd	nd	nd	nd	nd	0.262±0.001^{a}	0.098±0.01^{c}	0.15±0.04^{b}	tr	nd	tr	nd	nd	nd	nd
9	0.08±0.01^{a}	0.024±0.004^{c}	0.072±0.002^{b}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
10	nd	nd	nd	nd	nd	nd	tr	nd	tr	tr	nd	tr	nd	nd	nd	nd
11	nd	nd	nd	nd	nd	nd	0.061±0.001	nd	tr	tr	nd	tr	nd	nd	nd	nd
12	0.05±0.02^{a}	0.008±0.001^{c}	0.010±0.002^{b}	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
13	nd	nd	nd	nd	nd	nd	0.239±0.001^{c}	0.2±0.1^{d}	0.136±0.001^{f}	2.44±0.01^{a}	0.55±0.02^{b}	0.19±0.01^{d}	nd	nd	0.16±0.01^{e}	tr
14	0.041±0.001^{e}	0.050±0.001^{d}	0.003±0.0001^{f}	nd	nd	nd	0.47±0.01^{a}	0.109±0.003^{c}	0.35±0.02^{b}	nd	nd	nd	nd	nd	nd	nd
15	nd	nd	nd	nd	nd	nd	0.678±0.002^{a}	0.100±0.001^{g}	0.159±0.001^{d}	0.55±0.01^{b}	0.326±0.001^{c}	0.10±0.02^{g}	0.126±0.001^{f}	nd	0.20±0.03^{e}	nd
16	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.156±0.001	nd	nd	nd
17	nd	nd	nd	nd	nd	nd	2.2±0.1^{b}	nd	nd	3.4±0.1^{a}	0.95±0.01^{c}	0.27±0.01^{d}	nd	nd	nd	nd
18	nd	nd	nd	nd	nd	nd	0.49±0.02^{d}	0.48±0.01^{d}	1.13±0.03^{b}	1.31±0.01^{a}	0.53±0.02^{c}	0.22±0.01^{e}	nd	nd	0.18±0.03^{f}	nd
19	nd	nd	nd	nd	nd	nd	0.75±0.02^{a}	0.184±0.01^{c}	0.71±0.01^{b}	0.10±0.01^{d}	0.073±0.004^{e}	0.020±0.002^{f}	nd	nd	nd	nd
20	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.214±0.001	nd
21	nd	nd	nd	nd	nd	nd	0.32±0.01^{b}	0.107±0.001^{d}	0.17±0.01^{c}	0.38±0.01^{a}	nd	nd	0.11±0.01^{d}	nd	nd	nd
22	nd	nd	nd	nd	nd	nd	1.3±0.1^{a}	0.093±0.002^{d}	0.14±0.01^{c}	0.247±0.001^{b}	nd	nd	0.118±0.001^{c}	nd	nd	nd
23	nd	nd	nd	nd	nd	nd	0.83±0.01^{a}	0.19±0.01^{e}	0.39±0.01^{c}	0.76±0.01^{b}	0.254±0.003^{d}	0.113±0.004^{f}	0.11±0.01^{f}	nd	0.12±0.03^{f}	nd
24	nd	nd	nd	nd	nd	nd	0.67±0.02^{a}	0.176±0.001^{e}	0.31±0.01^{c}	0.451±0.004^{b}	0.21±0.01^{d}	0.084±0.003^{g}	0.14±0.01^{f}	nd	0.20±0.03^{d}	nd
TPA	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**5.1±0.1**^{a}	**1.214±0.01**^{e}	**1.443±0.003**^{d}	**4.33±0.02**^{b}	**1.929±0.003**^{c}	**0.61±0.01**^{f}	**nd**	**nd**	**0.579±0.002**^{g}	**0.231±0.003**^{h}
TF3O	**0.62±0.02**^{a}	**0.116±0.001**^{d}	**0.173±0.001**^{c}	**0.10±0.02**^{e}	**0.28±0.01**^{b}	**0.29±0.01**^{b}	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
TF	**0.17±0.01**^{f}	**0.037±0.001**^{j}	**0.081±0.002**^{h}	**nd**	**nd**	**nd**	**8.76±0.03**^{b}	**1.9±0.1**^{e}	**3.94±0.04**^{c}	**10.3±0.1**^{a}	**3.30±0.04**^{d}	**1.10±0.02**^{g}	**0.764±0.001**^{i}	**nd**	**1.09±0.02**^{g}	**nd**
TPC	**0.79±0.02**^{g}	**0.152±0.002**^{l}	**0.254±0.003**^{j}	**0.10±0.02**^{m}	**0.28±0.01**^{i}	**0.29±0.01**^{i}	**13.8±0.1**^{b}	**3.1±0.1**^{d}	**5.4±0.1**^{c}	**14.7±0.1**^{a}	**5.23±0.04**^{c}	**1.71±0.03**^{e}	**0.764±0.001**^{h}	**nd**	**1.66±0.02**^{f}	**0.231±0.003**^{k}

nd- not detected; tr- trace amounts; nq – not quantifiable; HyEth – Hydroethanolic extract; Inf - Infusion preparation; Dec- Decoction preparation. TPA- Total Phenolic Acids; TF3O- Total Flavan-3-ol; TF – Total Flavonoids; TPC- Total Phenolic Compounds. Standard calibration curves: quercetin-3-*O*-rutinoside ($y = 13343x + 76751$, $R^2 = 0.9998$, limit of detection (LOD) = 0.18 µg/mL and limit of quantitation (LOQ) = 0.65 µg/mL, peaks 5, 8, 10, 11, 13, 18, and 20); apigenin-6-*C*-glucoside ($y = 107025x + 61531$, $R^2 = 0.9989$, LOD = 0.19 µg/mL and LOQ = 0.63 µg/mL, peaks 7, 9, 12, and 14); quercetin-3-*O*-glucoside ($y = 34843x – 160173$, $R^2 = 0.9998$, LOD = 0.21 µg/mL and LOQ = 0.71 µg/mL, peaks 15, 16, 17, 19, 21, 22, 23, and 24); ferulic acid ($y = 633126x – 185462$, $R^2 = 0.999$, LOD = 0.20 µg/mL and LOQ = 1.01 µg/mL, peak 4); (+)-catechin ($y = 84950x – 23200$, $R^2 = 1$, LOD = 0.17 µg/mL and LOQ = 0.68 µg/mL, peaks 3 and 6); and *p*-coumaric acid ($y = 301950x + 6966.7$, $R^2 = 0.9999$, LOD = 0.68 µg/mL and LOQ = 1.61 µg/mL, peaks 1 and 2). In each row different letters mean statistically significant differences ($p<0.05$). *Mean statistical differences obtained by *t*-Student test.

Table 4

Antioxidant, anti-inflammatory and cytotoxic activities of hydroethanolic, infused and decocted extracts of *M. oleifera* edible parts.

		Seeds		Student's *t*-test	Flowers		Student's *t*-test	Fruits		Student's *t*-test
		Quinhamel	**Bissau**	*p*-value	**Quinhamel**	**Bissau**	*p*-value	**Quinhamel**	**Bissau**	*p*-value
Antioxidant activity*										
TBARS (IC_{50}, mg/mL)	Hydroethanolic	0.09±0.01c	0.09±0.01c	0.228	0.06±0.01c	0.07±0.01c	0.008	0.15±0.01b	0.14±0.01b	0.471
	Infusion	0.42±0.01b	0.92±0.01a	<0.001	1.23±0.02a	0.99±0.01a	<0.001	np	np	-
	Decoction	0.51±0.02a	0.82±0.02b	<0.001	1.06±0.06b	0.85±0.04b	<0.001	1.56±0.02a	1.49±0.05a	0.015
OxHLIA (IC_{50}, µg/mL)	Hydroethanolic	na	na	-	na	na	-	na	na	-
Δt = 60 min	Infusion	5.1±0.1b	2.4±0.2b	<0.001	17.0±0.6b	2.8±0.2b	<0.001	np	np	-
	Decoction	29±3a	29±2a	0.729	124±2a	89±2a	<0.001	265±7	55±3	<0.001
	Hydroethanolic	na	na	-	na	na	-	na	na	-
Δt = 120 min	Infusion	10.1±0.2 b	8.1±0.8 b	0.023	29±1 b	7.8±0.7 b	<0.001	np	np	-
	Decoction	101±4 a	109±4 a	0.079	222±2 a	160±3 a	<0.001	583±26	126±4	<0.001
Anti-inflammatory activity**										
NO-production inhibition	Hydroethanolic	208±14c	180±9c	0.015	>400	>400	-	>400	>400	-
(EC_{50}, µg/mL)	Infusion	230±9b	237±6a	0.153	>400	>400	-	np	np	-
	Decoction	248±4a	230±17b	0.006	>400	>400	-	>400	>400	-
Cytotoxicity to tumour cells***										
HeLa (GI_{50}, µg/mL)	Hydroethanolic	160±8c	173±6c	0.001	272±6	300±9	<0.001	>400	>400	-
(cervical carcinoma)	Infusion	201±16b	225±15b	0.272	>400	>400	-	np	np	-
	Decoction	229±3a	230±17a	0.854	>400	>400	-	>400	>400	-
HepG2 (GI_{50}, µg/mL)	Hydroethanolic	95±2c	82±5b	0.060	184±12	222±19	<0.001	> 400	> 400	-
(hepatocellular carcinoma)	Infusion	208±7b	224±14a	0.016	>400	>400	-	np	np	-
	Decoction	254±6a	224±17a	<0.001	>400	>400	-	>400	>400	-
MCF-7 (GI_{50}, µg/mL)	Hydroethanolic	167±7c	180±13b	0.001	163±5	187±10	<0.001	>400	>400	-
(breast carcinoma)	Infusion	202±8b	233±5a	0.001	>400	>400	-	np	np	-
	Decoction	251±7a	232±4a	0.004	>400	>400	-	>400	>400	-
NCI-H460 (GI_{50}, µg/mL)	Hydroethanolic	105±10c	129±15b	<0.001	245±9	271±13	<0.001	>400	>400	-
(non-small cell lung cancer)	Infusion	232±19b	239±4a	0.414	>400	>400	-	np	np	-
	Decoction	301±10a	239±6a	<0.001	>400	>400	-	>400	>400	-
Cytotoxicity to non-tumour cells***										
PLP2 (GI_{50}, µg/mL)	Hydroethanolic	327±8	347±7	0.075	>400	>400	-	>400	>400	-
(porcine liver primary culture)	Infusion	>400	>400	-	>400	>400	-	np	np	-
	Decoction	>400	>400	-	>400	>400	-	>400	>400	-

na - no activity; np - not performed. *IC_{50} values translate the extract concentration providing 50% of antioxidant activity (TBARS assay) or required to keep 50% of the erythrocyte population intact for 60 and 120 min (OxHLIA assay). Trolox IC_{50} values: 19.6±0.7 µg/mL (OxHLIA, Δt 60 min), 41±1 µg/mL (OxHLIA, Δt 120 min), and 23 µg/mL (TBARS), **EC_{50} values translate the extract concentration providing 50% of NO-production inhibition. Dexamethasone EC_{50} value: 16 µg/mL. ***GI_{50} values correspond to the extract concentration responsible for 50% of cell growth inhibition. Ellipticine GI_{50} values: 3 µg/mL (PLP2), 1 µg/mL (MCF-7), 1 µg/mL (NCI-H460), 2 µg/mL (HeLa), and 1 µg/mL (HepG2). In each column, for each variable, different letters correspond to significant differences between extracts ($p < 0.05$).

Table 5

Antibacterial and antifungal activity of hydroethanolic, infused and decocted extracts of *M. oleifera* edible parts.

		Seeds		**Flowers**		**Fruits**		**Positive controls**	
		Quinhamel	**Bissau**	**Bissau**	**Quinhamel**	**Quinhamel**	**Fruits Bissau**	**Streptomycin**	**Ampicillin**
Antibacterial activity (mg/mL)		MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC	MIC/MBC
B. cereus	Hydroethanolic	0.075/0.15	0.10/0.20	0.10/0.20	0.10/0.20	0.20/0.40	0.20/0.40		
	Infusion	0.075/0.15	0.60/0.90	0.15/0.30	0.15/0.30	np	np	0.04/0.10	0.25/0.45
	Decoction	0.075/0.15	0.037/0.075	0.075/0.30	0.075/0.30	0.20/0.30	0.15/0.30		
S. aureus	Hydroethanolic	0.10/0.20	0.25/0.50	0.30/0.60	0.30/0.60	0.20/0.40	0.20/0.40		
	Infusion	0.15/0.30	0.50/0.90	0.30/0.60	0.45/0.60	np	np	0.10/0.20	0.25/0.40
	Decoction	0.075/0.15	0.037/0.075	0.075/0.30	0.15/0.30	0.20/0.30	0.20/0.30		
L. monocytogenes	Hydroethanolic	0.10/0.20	0.45/0.90	0.10/0.20	0.10/0.20	0.10/0.20	0.10/0.20		
	Infusion	0.10/0.15	0.60/0.90	0.15/0.30	0.15/0.30	np	np	0.20/0.30	0.40/0.50
	Decoction	0.075/0.15	0.037/0.075	0.05/0.10	0.20/0.30	0.20/0.30	0.075/0.15		
E. coli	Hydroethanolic	0.10/0.20	0.10/0.20	0.075/0.15	0.10/0.20	0.10/0.25	0.10/0.20		
	Infusion	0.10/0.15	0.15/0.30	0.075/0.15	0.10/0.15	np	np	0.20/0.30	0.40/0.50
	Decoction	0.05/0.15	0.037/0.075	0.10/0.15	0.20/0.30	0.075/0.15	0.10/0.15		
E. cloacae	Hydroethanolic	0.10/0.20	0.30/0.60	0.30/0.60	0.50/1.00	0.30/0.60	0.25/0.50		
	Infusion	0.15/0.30	0.90/1.20	0.30/0.60	0.40/0.90	np	np	0.20/0.30	0.25/0.50
	Decoction	0.05/0.15	0.037/0.075	0.075/0.15	0.20/0.30	0.075/0.15	0.10/0.15		
S. Typhimurium	Hydroethanolic	0.10/0.20	0.30/0.60	0.10/0.15	0.30/0.60	0.25/0.50	0.15/0.30		
	Infusion	0.15/0.30	0.30/0.90	0.15/0.30	0.45/0.60	np	np	0.20/0.30	0.75/1.20
	Decoction	0.037/0.075	0.018/0.075	0.25/0.60	0.25/0.60	0.20/0.30	0.075/0.15		
Antifungal activity (mg/mL)								Ketoconazole	Bifonazole
		MIC/MFC	MIC/ MFC	MIC/ MFC	MIC/ MFC	MIC/ MFC	MIC/ MFC	MIC/ MFC	MIC/ MFC
A. fumigatus	Hydroethanolic	0.075/0.15	0.05/0.075	0.25/0.50	0.20/0.40	0.10/0.20	0.10/0.20		
	Infusion	0.075/0.15	0.05/0.10	0.30/0.60	0.075/0.15	np	np	0.25/0.50	0.15/0.20
	Decoction	0.018/0.037	0.075/0.15	0.018/0.037	0.075/0.15	0.075/0.15	0.075/0.15		
A. ochraceus	Hydroethanolic	0.075/0.15	0.075/0.15	0.015/0.030	0.075/0.15	0.10/0.20	0.10/0.20		
	Infusion	0.037/0.075	0.037/0.075	0.075/0.15	0.037/0.075	np	np	0.20/0.50	0.10/0.20
	Decoction	0.037/0.075	0.037/0.075	0.018/0.037	0.037/0.75	0.037/0.075	0.037/0.075		
A. niger	Hydroethanolic	0.037/0.075	0.037/0.075	0.15/0.30	0.15/0.30	0.30/0.60	0.30/0.60		
	Infusion	0.037/0.075	0.037/0.075	0.15/0.30	0.15/0.30	np	np	0.20/0.50	0.15/0.20
	Decoction	0.075/0.15	0.075/0.15	0.075/0.15	0.037/0.075	0.075/0.15	0.037/0.075		
P. funiculosum	Hydroethanolic	0.037/0.075	0.037/0.075	0.037/0.075	0.15/0.30	0.15/0.30	0.15/0.30	0.20/0.50	0.20/0.25

	Infusion	0.037/0.075	0.075/0.15	0.05/0.10	0.15/0.30	np	np		
	Decoction	0.037/0.075	0.037/0.075	0.037/0.075	0.075/0.15	0.075/0.30	0.037/0.075		
P. ochrochloron	Hydroethanolic	0.037/0.075	0.037/0.075	0.10/0.20	0.15/0.60	0.45/0.90	0.60/1.20		
	Infusion	0.10/0.15	0.15/0.30	0.075/0.15	0.20/0.40	np	np	2.50/3.50	0.20/0.25
	Decoction	0.075/0.15	0.075/0.15	0.075/0.15	0.30/0.45	0.075/0.15	0.037/0.075		
P. aurantioriseum	Hydroethanolic	0.037/0.075	0.037/0.075	0.15/0.30	0.30/0.60	0.10/0.20	0.15/0.30		
	Infusion	0.075/0.15	0.15/0.30	0.075/0.15	0.20/0.40	np	np	0.20/0.30	0.10/0.20
	Decoction	0.075/0.15	0.075/0.15	0.037/0.15	0.30/0.45	0.075/0.15	0.037/0.15		

MIC - minimum inhibitory concentrations; MBC - minimum bactericidal concentration; MFC - minimum fungicidal concentration; np - not performed.